International Journal of All Research Education and Scientific Methods (IJARESM),
ISSN: 2455-6211, Volume 12, Issue 12, December-2024, Available online at: www.ijaresm.com

Isolating Potassium Solubilizing Bacteria from different locations in Sri Lanka and Evaluating the Effect on Growth and Yield of Brinjal

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ABSTRACT

Potassium-solubilizing bacteria (KSB) based bio fertilizer is a sustainable approach to increase K availability in soil for crops. Present study was conducted to assess the ability of Potassium Solubilizing Bacteria inoculum (KSB) to enhance the growth and yield of Brinjal under plant – house condition. The KSB was isolated from six (06) soil samples collected from different locations in Sri Lanka; Matale Alfisol, Artisol, Jaffna Oxisol, Ultisol and Gannoruwa Ultisols. KSB is naturally present in Matale Alfisol and Jaffna Ultisol soils. Six (06) treatments were applied; Nitrogen (N), Phosphorous (P), Potassium (K), NP+K inoculum, NP+ 50%K+K inoculum, NP+75%K + K inoculum, NP only and No fertilizer. KSB inoculum as well as the K inorganic fertilizer application was increased the plant height, number of flowers and number of leaves per plant significantly ($p<0.05$). Further, Shoot and root biomass was increased significantly ($p<0.05$) with KSB application. However, highest growth was observed in NP+75%K + K inoculum treatment. Number of fruits per plant and fruit dry weight was increased significantly ($p<0.05$) by the KSB inoculum and K inorganic fertilizer applied treatments. The highest yield was observed in trial treated with both KSB and 75% K chemical fertilizer. The results indicated that KSB application increased soil available K. The best growth and yield promotion of Brinjal was achieved by applying 75% Potassium, Nitrogen and Phosphorus chemical fertilizer along with Potassium solubilizing bacteria.

Keywords: Bio Fertilizer, Potassium Solubilizing Bacteria, Inoculum, Alfisol, Ultisols, Artisols, Oxisols

INTRODUCTION

Plant nutrients which can be categorized as macronutrients and micronutrients are essential elements required for the growth of the plants. Potassium (K+) is indispensable for plant growth, metabolism, and development, regulating functions such as protein synthesis, enzyme activation, stomatal regulation, and photosynthesis, while also playing a critical role on abiotic stress responses (Singh et al., 2020; Kumar et al., 2022). K deficiency can severely impact plant health, leading to reduced crop yield and quality. Lack of potassium in the soil leads to poor development of roots, shoots, branches while having; slow growth, producing smaller seeds with lower yield (Rani & Sengar, 2020; Sustr et al., 2019). Feldspar and Mica are the common potassium supplement for soil (Rawat et al., 2016; Br & Jagadeesh, 2017). Moreover, due to intense application of chemical fertilizer, there is a lack of complete utilization of minerals by plants and excess of minerals are wasted away through rain water or excessive irrigation, this leads to shortage of potassium availability to plants (Zaarur & Erel, 2024; Chen et al., 2020).

In order to resolve these problems an alternate method of potassium availability to plants and maintaining the K level in soil has to be followed to achieve a higher yield without disturbing soil texture (Wakeel & Ishfaq, 2022; Bell et al, 2021). The recent research publications indicate the importance of maintaining Potassium balance in soil (Singh et al., 2022; Olaniyan et al., 2022; Jain et al., 2022; Naorem et al., 2021). Potassium Solubilizing Microorganisms (KSB) in soil helps to convert complex potassium minerals into simple potassium ion and provide them to plants (Adwani et al., 2024; Olaniyan et al., 2022; Yadav, 2022). Further, chemical fertilizer is identified as a major problem in current agriculture industry (Viswambharan & Kh, 2024; He et al., 2020; Tayoh, 2020) specially because of the misuse of the fertilizer. In order to achieve high production, farmers apply higher than recommended chemical fertilizers, which could have a negative impact on the environment. These facts indicate the requirement of strategies for sustainable K management in soil. Moreover, biofertilizers serve as a sustainable and environmentally favorable alternative to chemical fertilizers, as they increase the availability of potassium (K) nutrients in soil. (Jana et al., 2024; Sharma et al., 2024; Rehman et al., 2022). These natural

substances containing living microorganisms enhance the availability of nutrients to plants, support biodiversity and soil health in addition to enhancing nutrient uptake. Biofertilizers may efficiently solubilize potassium and other vital elements by developing symbiotic interactions with plants (Kalamulla & Yapa, 2024; Sharma et al., 2024; Olaniyan et al., 2022; Berde et al., 2021). This minimizes environmental degradation and lessens dependency on synthetic inputs. Several studies were conducted based on Potassium solubilizing bacteria-based bio fertilizers on the growth enhancement of the crops (Singh et al., 2022; Naorem et al., 2021; Pandey et al., 2020). These organic amendments not only improve soil health but also contribute to sustainable agricultural practices.

Brinjal (Solanum melogina**)** belong to the family of Solanaceae and genus Solanum was selected to evaluate the impact of KSB in the present study. The potassium fertilizer requirement for brinjal (Solanum melongena) varies based on soil conditions and targeted yields. Several researches indicate the importance of applying potassium for optimizing brinjal yield, with recommendations ranging from 30 to 90 kg K_2O per hectare depending on specific conditions and practices (Pradeepa et al., 2024; Rangaiah et al., 2023). The use of different biofertilizers in brinjal (Solanum melongena) cultivation has also shown significant benefits in enhancing growth, yield, and quality across various studies (Elayaraja et al., 2024; Chakravarthy & Mohan, 2023; Prasad et al., 2023). The main objective of the present study was to determine the effect of Potassium solubilizing Bacteria on growth and yield of brinjal under Sri Lankan contest. We investigate the effect of potassium Solubilizing Bacteria along with different rate of inorganic fertilizer on growth and yield of Brinjal under six different types of soil from three districts in Sri Lanka.

MATERIAL AND METHODS

Location of the Experiment

The experiment was conducted at the Division of Soil Microbiology, Horticulture Crop Research and Development Institute (HORDI), Gannoruwa, Peradeniya under controlled environmental condition in a plant house.

Isolation of potassium solubilizing bacteria (KSB) from soils of different locations in Sri Lanka

Six Soils samples were collected from Matale (Alfisol, Artisol), Jaffna (Oxisol, Ultisol) and Kandy (Ultisol) (Table 01). The soil samples were randomly collected from each sampling site in triplicates at a depth of 6 cm from the surface, packed in zip-lock bags, brought into the lab, and stored at −4◦C until further use.

Table 01: Details of the Soil samples

Location	Districts	GPS coordinates	USDA soil type	Great soil group
Matale town	Matale	7.472797,80.621421	Artisols	Sandy Regosls
Maruthnarmadam, Jaffna	Jaffna	9.7311020,80.0267160	Oxisols	Red Latasol
Point Petro, Jaffna	Jaffna	9’4912.1’’N80’1348.2’’E	Ultisols	Red yellow Latasol
Palapatwala	Matale	7.5247430,80.6302810	Alfisol	Sandy Regosol
HORDI, Gannoruwa	Kandy	7.276221,80.597051	Ultisols	Reddish brown Podsolic
HORDI organic field, Gannoruwa	Kandy	7.245632,79.607053	Ultisols	Reddish brown Podsolic

This selective growth medium supports the growth of KSB by providing the necessary nutrients while inhibiting non-target organisms. Count the colonies in each plate after incubating the inoculated plates for 72 hrs at 30°C. The isolated colonies were grown in Aleksandrow broth for 72 hours (Akasah et al., 2024; Adwani et al., 2024), cross streaked in Aleksandrow media and incubated for 72 hours to obtain the pure colonies. The isolation of potassium solubilizing bacteria (KSB) from fresh soil samples using the dilution plate technique on Aleksandrov media is a well-documented method (Adwani et al., 2024; Bhalani & Dasgupta, 2024; Bangun et al., 2023) that facilitates the identification of effective microbial agents for enhancing potassium availability in soils. Pure colonies were used as inoculum in treatments.

Preparing the pots for treatment applications

The potted soil (ultisols) was obtained from the research field in HORDI, Gannoruwa, Initial investigations were carried out by estimating the pH, EC, Exchangeable K and organic matter from the randomly collected soil samples from the same filed. The 1 ft diameter pots were filled with 5 kg of well-sized soil. Commercially available seeds of Brinjal (variety; HORDI Lena Iri) were sown on a nursery to use as the planting material. Four (04) weeks old healthy, vigorous, and well grown seedlings were transplanted into pots applying six (06) treatments with five (05) replicates.

Experimental design and treatment combinations

The experiment was laid out in Randomized Block Design. The experiment consist of isolated KSB and five (05) level of inorganic fertilizers as treatments are applied as soil application. The treatments comprised of T1 – Department of Agriculture in Sri Lanka recommended fertilizer mixture: Urea as the Nitrogen (N) source, Tripple Super Phosphate (P) as the Phosphate source and the Muriate of Potash (K) as the potassium source, T2 – NP + Inoculum (50 ml), T3 – NP + 50% K + Inoculum (50ml) T4 - NP+75%K+Inoculum (50ml) (K), T5 – NP only and T6 – No added fertilizer. KSB inoculum was inoculated to Brinjal two (02) days after transplanting. T2, T3 and T4 were inoculated with 50 ml of inoculum containing approximately $2.7\text{-}3.0\times10^{11}$ CFU/ml colony count. Inoculation was repeated in every month until four (04) months. Growth and yield parameters of Brinjal were collected from all treatments.

Statistical Analysis

The data were statistically analyzed using the Analysis of variance (ANOVA) and the significance of the difference among the treatment combinations means were estimated by the Duncan's Multiple Range Test (DMRT) at $P<0.05$ level of probability using SPSS 26 statistical package.

RESULT AND DISCUSSION

Soil Characterization

The results of the initial soil analysis were given in Table 2. The Soil pH of the studied soil was 6.7 ± 0.04, thus soil was within the neutral range. The exchangeable K of studied soil was low (117.64 ± 0.2 ppm). Further, organic matter content and the electrical conductivity were appeared to be low as the 3.7 ± 0.2% and 102.3 ± 0.8 ds/m respectively, thus it is non saline soil (Mapa et al. 1999).

Table 2: Initial Soil Characterization

Chemical parameters	Value
pH	6.7
EC (ds/m)	102.3
Exchangeable potassium	117.64
Organic matter (%)	3.7

Estimating the potassium solubilizing bacteria availability in different soils

Table 3 explains the availability of potassium solubilizing bacteria in collected soils. Highest amount of Potassium solubilizing bacteria was reported in Palapatwala (5.4×10^4 CFU/ml) [Alfisols (Sandy Regols)] and Point Petro soil (6.9×10^4 CFU/ml) [Ultisols (Red yellow latasols)]. The nature of the ultisols soil was clay but not sticky which contain 20% organic matter having high range of exchangeable K^+ and with soil acid (Meena, 2014). Further according to the findings of Meena in 2014 soil organic acid and exchangeable K^+ ion influence more on the Potassium Bacteria solubilizing effect. Alfisols soil has a high concentration of nutrient cation K^+ and it contains $CaCO_3$ (Meena, 2014). According to researcher Sheng (2005) high K^+ cation influence more Potassium Bacteria Solubilizing effect and calcium carbonate induces more Potassium solubilization.

Table 3: Potassium Solubilizing Bacteria Content In Selected Soils.

Location	USDA soil classification	Colony count (CFU/ml)
Matale	Artisols	0.2×10^4
Marthanarmadam	Oxisols	0.7×10^4
Point petro	Ultisols	6.9×10^4
Palapathwala	Alfisols	5.4×10^4
HORDI Gannoruwa	Ultisols	1.2×10^4
HORDI organic field, Gannoruwa	Ultisols	1.4×10^4

Effect of Potassium Levels and Potassium Solubilizing Bacteria on Plant height of Brinjal.

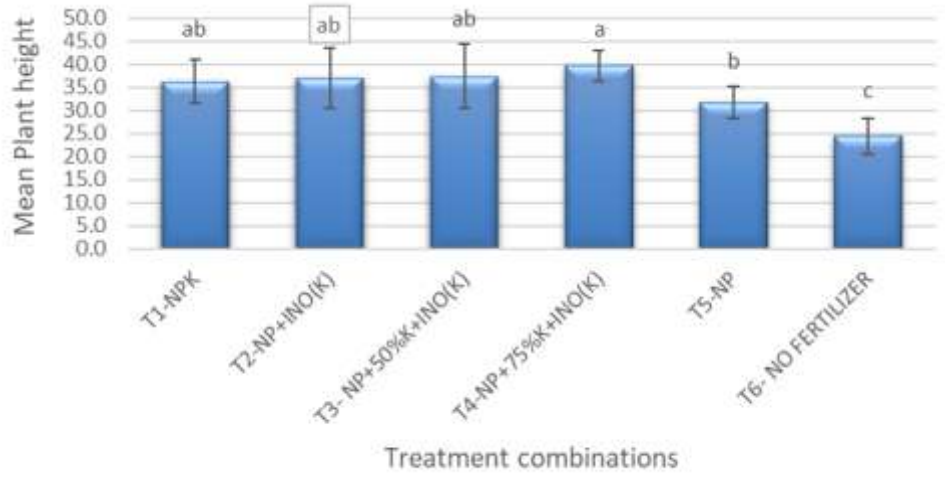

Figure 01: Effects of Potassium Levels and Potassium Solubilizing Bacteria on Plant height of Brinjal. Treatment with different letters is not significantly different ($p<0.05$)

Figure 01 explain the effects of potassium levels and potassium solubilizing bacteria on plant height of brinjal. There was no significant difference in plant height among treatments T1, T2, T3 and T4. However, the highest plant height (39.80 ± 1.2 cm) was observed in trial treated with both KSB and 75% K inorganic fertilizer, whilst the lowest height (24.3 ± 0.4 cm) was observed in treatment T6, without fertilizer. According to the findings of Han and Lee (2005) inorganic K fertilizer application did not significantly increase the plant growth. Increase in plant growth was occurred in the combined treatment; soil inoculated with K Bacteria and inorganic K fertilizer. AS per the studies done by Sheng in 2005 the plant growth promoting effects and nutrient uptake level was influenced by Potassium solubilizers. Similarly, K solubilizing bacteria increase the plant height as well as the K uptake in Brinjal as per the research conducted by Ramerethinam and Krishnan in 2006.

Effect of Potassium Levels and Potassium Solubilizing Bacteria on Number of leaves, flowers and buds per plant

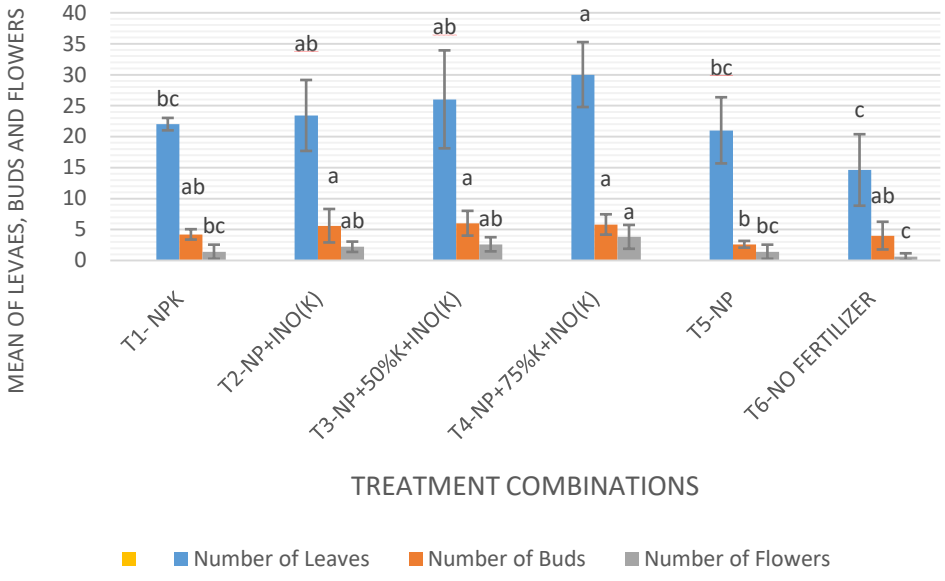

Figure 02: Effect of Potassium Levels and KSB on number of leaves, number of flower and number of buds. Treatment with different letters are not significantly different ($p<0.05$)

Figure 02 explain the effect of potassium levels and KSB on number of leaves, number of flower and number of buds. The highest number of leaves (30 ± 5.2) and buds (5 ± 1.6) were observed in T4 which was treated with 75% inorganic K fertilizer along with K inoculum. T4 was statistically identical to T2 and T3. Potassium Bacteria inoculum which influences to increased leaf and bud rate, enhance the early growth in plants and had increased the increase photosynthesis. (Han and Lee 2005). Number of flowers was significantly higher in treatment T4 (3.80 ± 1.9) when compared with un-inoculated treatments (T1, T5 & T6). Plant flowering and the early flowering rates were influenced by the Potassium solubilizing Bacteria (Nayak, 2001). According to the findings of Han and Lee (2005) that K chemical fertilizer application was not significantly increase the plant flower and leaves rate. An increase of plant flower rate was occurred in the combined treatment, i.e., soil inoculated with both K Bacteria and K chemical fertilizer.

Effect of Potassium Levels and Potassium Solubilizing Bacteria on Root length and the Root Fresh Weight

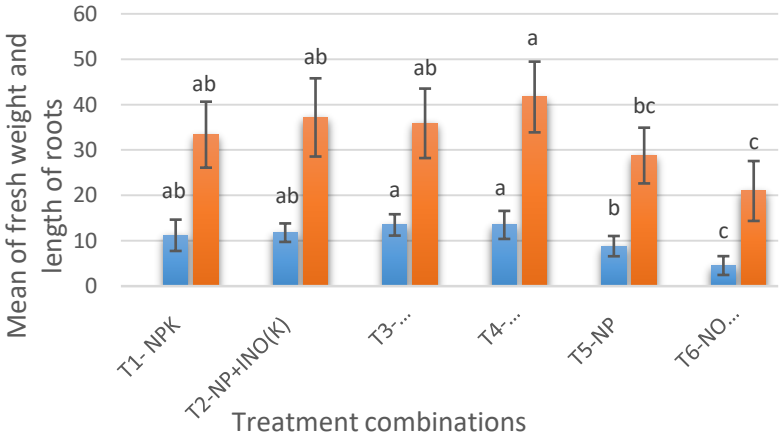

Figure 03: Effects of Potassium Levels and KSB on root fresh weight and root length. Treatments with different letters are not significantly different (p<0.05)

As per the figure 03 the highest root fresh weight was observed in treatment T4 (13.52 ± 3 g) which was statistically identical to the T3 (13.52 ± 0.32 g), whilst the lowest fresh weight (4.57 ± 2.7 g) was observed in treatment T6, with no fertilizer added. The best root weight was observed in treatments T3 and T4 which were treated with both K chemical fertilizer and Potassium Bacteria inoculum. Combined application of Potassium inorganic fertilizer and KSB influences on increasing the root weight (Han and Lee 2005). The highest root length was observed in treatment T4 (41.7 ± 4.8 cm) added with 75% K inorganic fertilizer and K inoculum. As per the previous researchers (Singh et al. 2010; Sindhu et al. 2012), the enhancement of plant K nutrition might be due to the stimulation of root growth or the elongation of root hairs by specific KSB microorganisms. According to the Chandra et al., (2005) the plant shoot and root promoting effects and nutrient uptake level were influenced by Potassium solubilizers.

Effect of Potassium Levels and Potassium Solubilizing Bacteria on Dry Biomass

As per the Table 04 dry biomass was significantly higher ($p<0.05$) in all inoculated treatments compared to un-inoculated treatments. The highest dry biomass (18.8256 ± 2.7 g), dry shoot weight (13.1522 ±1.7 g) and dry root weight (5.6734±1.31 g) was observed in treatment T3. Nayak (2001) investigated the effect of K mobilizer on brinjal and recorded increased K uptake and increased plant biomass in mobilizer treated plant as compared to un-inoculated.

Table 04: Effect of Potassium Levels and Potassium Solubilizing Bacteria on Dry Biomass

Root dry weight	Mean root dry weight	Mean shoot dry weight	Mean dry biomass
T1- NPK	4.2804 ± 1.1^{a}	9.7102 ± 1.31^{ab}	11.7256 ± 2.29^{b}
T2- NP+ K inoculum	5.4072 ± 1.38^{a}	12.481 ± 2.4^{a}	17.8882 ± 3.47^{a}
T3- NP+50%K+ K inoculum	5.6734 ± 1.31^{a}	13.1522 ± 1.7^{a}	18.8256 ± 2.74^{a}
T4- NP+75%K + K inoculum	5.1068 ± 1.5^{a}	12.481 ± 3.3^{a}	17.5878 ± 2.7^{a}
T5- NP	2.4430 ± 0.9^{b}	7.8686 ± 1.297^{b}	10.3116 ± 2.07^{b}
T6 – No fertilizer	2.2424 ± 1.04^{b}	6.7742 ± 1.9^{b}	9.0166 ± 2.87^{b}

Effect of Potassium Levels and Potassium Solubilizing Bacteria on yield and yield determining parameters

As per the figure 04 number of fruits per plant was significantly higher ($p<0.05$) in treatment T3 (2 ± 0.7) and T4 (2.6 ± 1.1) which was treated with KSB bacteria as well as K inorganic fertilizer. Studies on the effect of K solubilizing bacteria Frateuria aurantia on Brinjal showed a significant increase in yield and K uptake as per the Ramarethinam and Krishan in 2006. The inoculation of KSB along with chemical K fertilizer into the soil increased K uptake, photosynthesis and the yield of eggplant grown on K limited soils (Sangeeth, 2012).

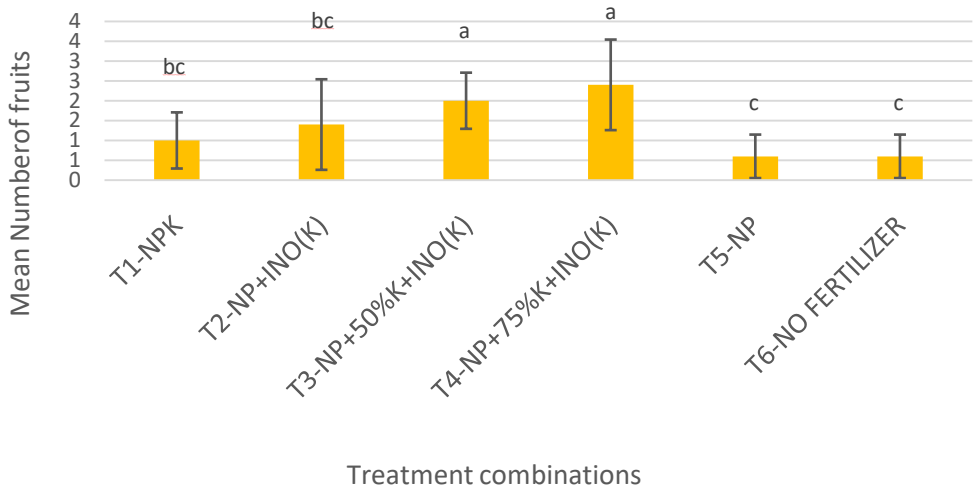

Figure 04: Effects of treatments on number of fruits. Treatments with different letters are not significantly different ($p<0.05$)

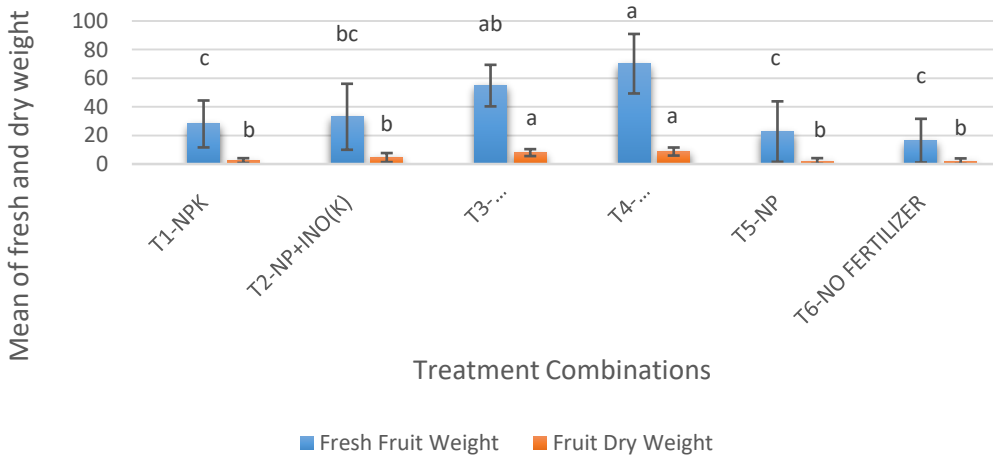

Figure 05: Effects of treatments on fruit fresh and dry weight. Treatments with different letters are not significantly different ($p<0.05$)

The highest fresh weight of the fruits was observed from treatment T4 (70.177 ± 7.78 g) which was statistically identical to the T3 (54.83 ± 4.5 g) Dry weight of the fruit was significantly high in T3 (8.02 ± 2.4 g) and T4 (8.78 ± 2.8 g) which was treated with both K chemical fertilizer and K inoculum. NPK- applied treatment was shown approximately the same fruit growth compare with K inoculum and NP applied treatment. According to the Archana et al. (2013), the K-solubilizing bacteria, Showed an increase yield in maize. That indicates the KSMs significantly increased yield, plant growth, and nutrient uptake component over absolute fertilizer control. Badr et al. (2006) reported that the application of KSB with K minerals on sorghum enhanced the dry matter yield by 48 % and 65 % respectively.

Abundance of potassium solubilizing Bacteria in soil

T4 (5.4×10^7) soil was contained high potassium solubilizing bacteria month after the transplanting, however at the end of the experiment the abundance of the potassium solubilizing bacteria was increased by 79% when compared to abundance

of bacteria during its first month. T3 was contained (2.94×10^8) highest KSB concentration at the end of the experiment. Inoculated treatments T2, T3 and T4 soil was induced higher bacteria abundance at the end of the trial compared to the bacteria abundance during its first month. According to the findings of Hu et al., (2006) that the continuous application of inoculum increased the sustainability of Bacteria in soil. Studies was shown that inoculating soil with KSB boosts the growth of rye and its absorption of N and K (Basak and Biswas, 2010). KSB was found to dissolve slow-release K compounds, which increased the concentration of available K in the soil, further facilitating the plant growth. Correlation analysis showed that the tobacco biomass and K content were positively correlated with soil K ion content (Zhang, and Kong, 2014).

Table 04: Abundance of potassium solubilizing bacteria in soil

Treatments	Initial (CFU/ml)	1st month after transplanting (CFU/ml)	End of the experiment (CFU/ml)
T1 - NPK	0.2×10^7	0.4×10^7	0.6×10^7
T2 – NP + INO (K)	0.2×10^7	2.9×10^7	0.3×10^9
T3 – NP + 50%K +I NO (K)	0.2×10^7	5.0×10^7	2.94×10^8
T4 – NP + 75%K + INO (K)	0.2×10^7	5.4×10^7	2.64×10^8
T5 - NP	0.2×10^7	0.1×10^7	0.1×10^7
T6 - NO FERTILIZER	0.2×10^7	0.2×10^7	0

CONCLUSION

As per the results of the present study, the highest natural abundance of potassium solubilizing bacteria was observed in the soils of Matale Alfisol and Jaffna Ultisol. The highest growth and yield promotion of Brinjal was achieved by the application of 75% of potassium, nitrogen, and phosphorus inorganic fertilizer and KSB inoculum. Further, inoculum applied treatment showed high plant dry biomass than the un- inoculated treatments. However, NPK applied treatment showed approximately the same growth and yield compared to the K inoculum and NP applied treatment. These results conclude that the ability of using K inoculum as an alternative to the potassium nutrient. This study will lead in reducing the inorganic fertilizer usage and also it increases potassium solubilizing bacteria abundance in soil. Therefore, potassium solubilizing bacteria inoculum can be considered as an environmentally friendly, safe and non-toxic-based fertilizer to use in vegetable cultivation. However, further research will be able to study more on the potassium solubilizing bacteria inoculum.

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